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Arti Arya
Department of Biotechnology, D.A.V. College, Sector 10, Chandigarh, India

Correspondence
Arti Arya
Department of Biotechnology, D.A.V. College, Sector 10, Chandigarh, India

Antibacterial property of laboratory preparations of garbage enzyme

Arti Arya

Abstract
Garbage enzyme is another name for Kitchen waste liquid ferments, produced as a result of 3 month fermentation process involving natural and mixed microbial cultures in the starting material (fruit and vegetable peels). These are reported to be eco-friendly enzyme preparations with multiple applications as household Cleaning solution, sanitizer, deodoriser, insect repellent, fertilizer, soil conditioner, and natural pesticide. In the present study, indigenous preparations of garbage enzyme, using two different types of organic waste sources, were produced and analysed for their antimicrobial activity against test microorganisms. Four bacterial and fungal strains were used for assay of antimicrobial activity using spread plate method. Results, obtained as zone of inhibitions, indicated significant antimicrobial activity against most of the test strains. This study provides evidence to the purposed application of garbage enzyme as cleanser and sanitizer. Garbage enzyme is suggested to provide a cheaper and eco-friendly alternative to commercially applied counterparts.

Keywords: Garbage enzyme, kitchen waste, antimicrobial activity

Introduction
Garbage Enzyme (GE) or fermented waste juice is a liquid concentrate of complex composition and is a product of 3-month fermentation of organic waste (fruits and vegetables peels).Garbage Enzyme was first reported by Dr. Rosukon Poompanvong, an alternative health-care practitioner from Thailand [1]. The ferments generally have a high acetic acid content along with variable amounts of alcohol, sugars, proteins, lipids, certain enzymes. Yeast and some other microbes are proposed to be present in some preparations [2]. It is claimed to be a multipurpose solution for household and agricultural uses and is reported to act as household cleanser, insect repellent, disinfectant, soil conditioner, fertilizer, pesticide etc. [3]. It has been suggested that indigenous GE preparations may have antimicrobial properties contributed by acetic acid and ethanol components. Large scale GE production offers a green and eco-friendly approach to manage organic wastes generated in huge amounts from both households as well as industrial sources. In this context, the present work reports the antimicrobial activity of Garbage enzyme preparations against arbitrarily chosen bacterial and fungal strains.

Material and Methods
Microbes Used
Bacterial strain-*E. Coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*

Method for GE preparation [4]
A Ratio of 1:3:10 of sugar (jaggery/Molasses), vegetable/fruit peels and water was used. Air tight plastic bottles was used to allow expansion. These bottles were then placed in a cool dry and well-ventilated area. Direct sunlight was avoided. Gases were released daily from bottles. After 3 months, filtered the concentrated liquid from the residues and used it. GE-1: contained all fruits peels; GE-2: contained only vegetables peels.

Preparation of working culture [5]
Lyophilized culture of bacterial strains-*Escherichia coli, Pseudomonas, Bacillus subtilis, Staphylococcus* were procured from the Institute of Microbial Technology (IMTECH)

Chandigarh. The strains were procured as a slant culture. Nutrient Broth was inoculated with the bacterial strains and used.

0.5 McFarland Turbidity Standard [6]

0.5 McFarland Turbidity Standard is used to adjust the turbidity (concentration) of the inoculum for antimicrobial activity. The 0.5 McFarland turbidity standard was prepared by adding barium chloride to sulfuric acid. The mixture of the two chemicals forms a precipitate that when in suspension is equivalent approximately 1.5×10^8 colony forming units/ml.

Tests for Antimicrobial Activity by Agar Spread Plate Method [7, 8]

Antimicrobial activity of GE preparations was studied by Agar spread plate method and zones of inhibition were observed. Ampicillin (1mg/ml) was used as positive control for antibacterial assay and distilled water was used as negative control.

Antibacterial Activity Disc Diffusion Method [9]

0.1ml of each culture (*E. coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus*) was spread on individual Nutrient agar plates, aseptically. Each of bacterial culture was diluted to match 0.5 McFarland turbidity standards.After that, 60µl of sample was dispensed onto the stack of discs (8-10) (Whatman filter paper1 was used to make disc and these were uniformly cut with the help of paper puncher) and was gently pressed to agar using a flame- sterilized forceps. Plates were kept at 37 ºC for 24hrs to observe the zone of inhibition and diameters were recorded.

Statistical Analysis [10]

Results were represented as mean ±S.D. of 4-6 independent observations. Statistical Analysis was done for proper interpretation of the results.

Results and Discussion

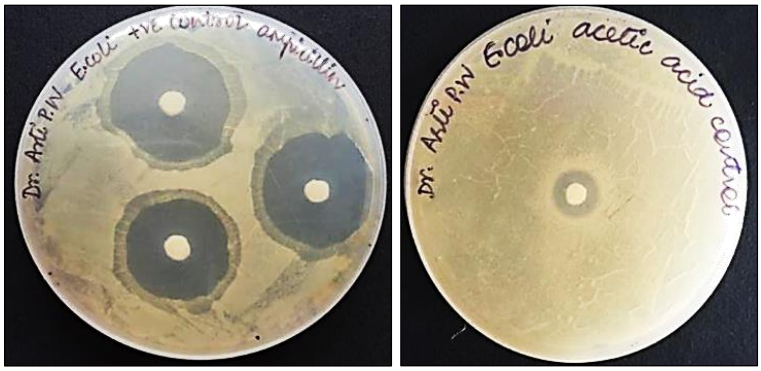
Antibacterial activity

Antibacterial activity of GE preparations was investigated with reference to acetic acid (2%) since acetic acid was found to be a major constituent.GE-1 showed better antimicrobial activity against *E. coli* than GE-2 as indicated by the zone of inhibition. The antimicrobial activity observed with 2% acetic acid and GE-2 were found to be comparable as both showed the zone of inhibition of ~1.7cm. It is suggested that acetic acid in GE might be primarily responsible for its antimicrobial activity [11]

Table 1: Antibacterial activity of GE against *E. coli*

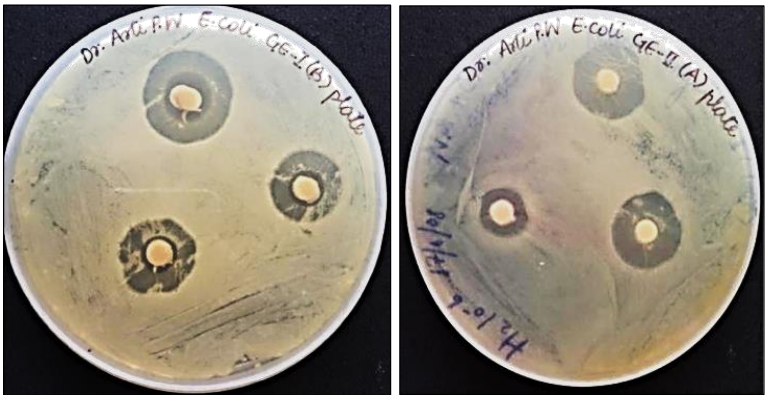
Sample	Zone of inhibition (cm)
Positive control (Ampicillin)	3.06±0.094
Acetic acid control (2%)	1.7±0.03
GE-1	2±0.163
GE-2	1.76±0.33
Negative control (water)	ND

Results are Mean ±SD OF 3-5 independent observations



(a) (b)

Fig 1(a, b): Showing the antibacterial activity in positive control and acetic acid.



(a) (b)

Fig 2 (a, b): Antibacterial assay of GE against *E. coli*

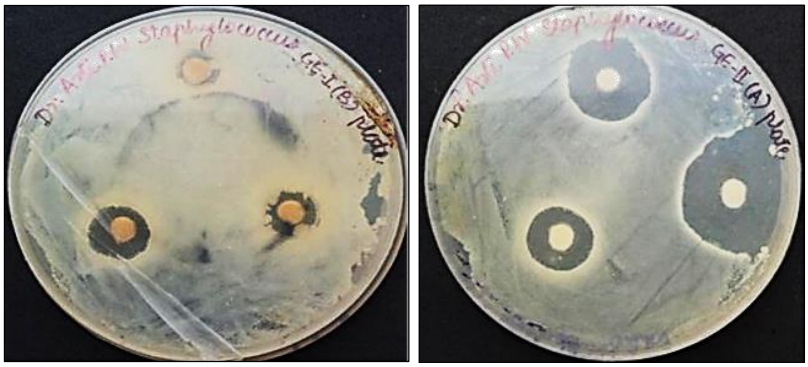
Table 2: Antibacterial activity of GE preparations against *Staphylococcus aureus*.

Sample	Zone of inhibition(cm)
Positive control (Ampicillin)	2.6 ±0.36
Acetic acid control	2.0±0.16
GE-1	1.23±0.188
GE-2	2.3±0.50
Negative control (water)	ND

Results are Mean ±SD OF 3-5 independent observations

The zone of inhibition diameter obtained with GE used for antibacterial assay against S. aureus showed that GE-2 inhibited the growth of *Staphylococcus* more efficiently as compared to GE-1 as shown in figure 3 (a, b). The antimicrobial activity of 2% acetic acid which was used as positive control were found to be intermediate between GE-1 and GE-2 which indicates towards the possibility that some other factors in GE may also have a role in influencing the growth of *Staphylococcus* other than acetic acid.

The antimicrobial activity of GE-2 was quite significant compared to ampicillin (positive control) as observed by the zone of inhibition.



(a) (b)

Fig 3 (a, b): Showing the antibacterial activity of GE1 and GE2 against *Staphylococcus aureus*

Table 3: Antibacterial activity of GE against *Bacillus subtilis*

Sample	Zone of inhibition (cm)
Positive control	3.1±0.141
Acetic acid control	1.0±0.12
GE-1	0.56±0.01
GE-2	0.82±0.03
Negative control	ND

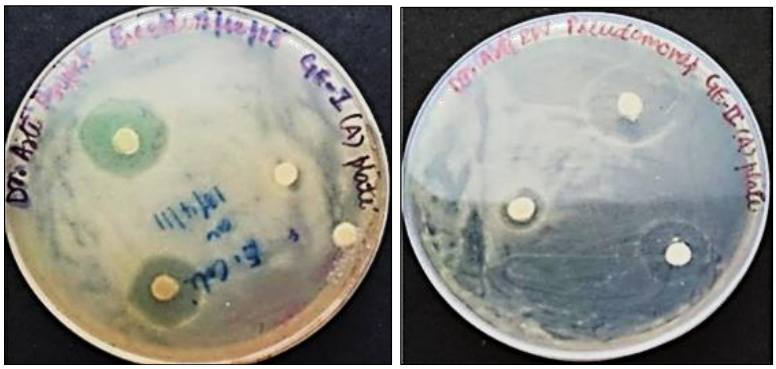
Results are Mean ±SD OF 3-5 independent observation

From the facts in table and figures below, it was cleared the acetic acid in GE was mainly responsible for the inhibition of *B. subtilis.* The diameters of zones of inhibition of GE 1 and GE 2 were comparable.

Table 4: Antibacterial activity of GE against *Pseudomonas aeruginosa*

Sample	Zone of inhibition (cm)
Positive control	1.83±0.235
Acetic acid control	1.7±0.56
GE-1	1.2±0.85
GE-2	1.76±0.531
Negative control	ND

As observed, antimicrobial activity of GE-2 may be due to the presence of acetic acid in it as both showed the zone of inhibition of ~1.7cm. The antimicrobial activity of GE-1 is less significant than GE-2.



(a) (b)

Fig 4 (a, b): Showing the antibacterial activity of GE1 and GE2 against *Pseudomonas aeruginosa*

Results obtained on the antimicrobial action of garbage enzymes may act as supporting evidence on the purposed role of garbage enzyme as multipurpose disinfectant and deodorant. Antimicrobial activity of garbage enzymes might be due to the bioactive constituents released from the vegetable peels and kitchen waste before and after fermentation. Same types of constituents are also present in plants which have many herbal and natural extracts where the proposed bioactive constituents have been some secondary metabolites, alkaloids or certain lipophilic compounds being secreted by plants or plant parts [12, 13].

Proposed applications of garbage enzyme as a multipurpose domestic cleanser and disinfectant, find evidence in the present study to some extent. Although the results are based on preliminary, it apparently provides data supporting the existing claims on the GE being a miracle liquid.

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